Serum Activin A and Inhibin A

New Clinical Markers for Hydatidiform Mole

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BACKGROUND. Although human placenta is a well established, rich source of proteins, hCG is the only measurement available to date in diagnosing the occurrence of the hydatidiform mole. Serum levels of a new placental protein, immuno reactive inhibin, were high in molar pregnancy, but the inhibin assay never became of clinical use, due to its low specificity and reliability. Since specific assays are now available for inhibin A, inhibin B, and activin A, the current study evaluated whether and which of these placental proteins is increased in presence of a molar pregnancy.

METHODS. Serum inhibin A, inhibin B, activin A, and hCG levels were assayed in: A) 6 women with molar pregnancies, before and after evacuation; B) 37 healthy pregnant women; and C) 22 healthy nonpregnant women.

RESULTS. Women with partial hydatidiform moles had significantly higher serum levels of inhibin A (P < 0.001) and activin A (P < 0.001) than healthy pregnant women, several fold higher than the 95% confidence interval of control values. After evacuation, the levels of both inhibin A (P < 0.001) and activin A (P < 0.05) declined significantly to the levels of nonpregnant controls. Molar hCG concentrations were significantly higher than in normal pregnancy (P < 0.001), but some values within the 95% confidence interval of normal values. Despite a significant decrease (P < 0.05) after evacuation, hCG levels were still higher than in nonpregnant women.

CONCLUSIONS. The present data strongly suggest that serum inhibin A and activin A measurement may be of value in diagnosis and short-term follow-up of molar pregnancy.

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Partially hydatidiform mole is a triploid gestational trophoblastic tumor, occurring in about 3 per 1000 pregnancies.1,2 The measurement of hCG is of great relevance for establishing the diagnosis, determining the response to chemotherapy, and detecting the rare recurrences.1,2 However, the initial hCG estimation is not definitive in differentiating between a normal and a molar pregnancy, since the quantity of hCG produced by a normal pregnancy can vary over a wide range.2 As a consequence, partial hydatidiform mole is rarely diagnosed in very early pregnancy only according to hCG values; it is considered when the ultrasound evaluation reveals a mixed pattern of echogenicity.1,2

In the last decade it has been shown that the human trophoblast produces and secretes several proteins/growth factors,3 and some of these (e.g., vascular endothelial growth factor, macrophage colony-stimulating factor, parathyroid hormone-related protein, SP1, PP5) are hypersecreted when hydatidiform moles occur.4–7 However, mea-
measurements of these were not useful in diagnosing trophoblastic disease because their serum levels were frequently not elevated when hCG concentrations were already significantly high. Establishing a reliable assay proved another difficulty.2

Recently, serum immunoreactive inhibin levels were found to be high in women with hydatidiform mole,3,9 which suggested these as a reliable tumor marker. However, that assay was never available in a large scale, was of poor reliability, and was not specific enough to distinguish between the various forms of inhibin-related proteins.3,10 In fact, during normal pregnancy, the human placenta secretes inhibin-related proteins (inhibin A, inhibin B, and activin A), a group of dimeric glycoproteic growth factors,3,11,12 into maternal serum, where they are measurable in high levels until term gestation.3,10

The aim of the current study was to establish, by means of a new assay, whether inhibin A, inhibin B, and activin A are hypersecreted and to determine the potential clinical value of their serum measurements in women with partial hydatidiform moles.

MATERIALS AND METHODS
A group of six women (mean age, 27 years; range, 19-36) were admitted for early pregnancy bleeding. Multiple serum hCG measurements (performed three times with intervals of at least one day) and ultrasound scans (USS; ELEGRA Millenium Edition, with a transvagal probe at 4.5-7.0 MHz, Siemens Sonoline; Erlangen, Germany) identified the hydatidiform mole; the histologic appearances of samples obtained at curettage confirmed the diagnosis. None of the patients had a previous history of molar pregnancy before, and gestational ages (4 patients at 12 weeks of pregnancy and 2 at 14 weeks) at the time of primary evacuation were calculated from the data of the last menstrual period and confirmed by USS. Blood specimens were also collected 10–15 days after evacuation of the molar pregnancy for hCG monitoring. The partial molar pregnancies were evaluated over a two year period from 1999 to 2000.

In addition, blood samples were also collected in two groups of control healthy women: 37 women at early pregnancy, from 12 to 14 gestational weeks, who progressed to deliver healthy single babies. Women with multiple pregnancies, diabetes, hypertension, fetal anomaly, or maternal or fetal infections were excluded; and twenty two nonpregnant women, enrolled during the follicular phase of the menstrual cycle, as assessed by the last menstrual period and confirmed by USS.

Blood was collected from the antecubital vein and allowed to clot. After centrifugation, serum was divided into several aliquots and stored at −20 °C until assay. Informed consent was obtained from each pregnant woman, and the permission of the local Human Investigation Committee was granted for the study.

Inhibin A, Inhibin B and Activin A Assay
Maternal serum inhibin A, inhibin B, and activin A concentrations were measured using specific two-site enzyme immunoassays (Serotec, Oxford, UK), as previously described.13 Briefly, plates were washed and bound alkaline phosphatase was quantitated by using a commercially available enzyme immunoassay amplification system (Immuno Select ELISA Amplification System, Dako, Milan, Italy), which was used according to the supplier’s instructions.

The inhibin A detection limit was 4 pg/mL in serum, with intra- and inter-assay coefficients of variation (CVs) for quality control samples of 4.0% and 8.0%, respectively. The inhibin B detection limit was less than 10 pg/mL, and within- and between-plate CVs were less than 6.0% and 8.0%, respectively. The limit of detection for activin A was less than 100 pg/mL, and intra- and inter-assay CVs were 5.0% and 9.0%, respectively. Cross-reactions for each assay with the various inhibin-related proteins were less than 0.5%.

Inhibin A, inhibin B, and activin A plates were read at 490 nm on an automated enzyme-linked immunosorbent assay (ELISA) plate reader (Basic Radim Immunoassay Operator [BRIO]; Radim spa, Pomezia, Italy).

hCG Assay
Maternal serum hCG levels were assayed with an IMMULITE Analyzer, by using a commercial diagnostic kit (EURO/DPC Ltd, Llanberis, UK). This assay has a sensitivity of 1.1 mIU/mL, a within-assay CV of 2.1%, and an inter-assay CV of 3.1%. The assay was highly specific for hCG, with no cross-reactivity luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH).

Statistical Analysis
The Mann—Whitney U test was used to evaluate the statistical significance of hormone changes between healthy controls and molar pregnancies before evacuation, as well as between follicular phases and values after molar evacuation. The Friedman test followed by post hoc Dunn test was used for comparison of multiple paired samples before and after evacuation. Statistical significance was assumed whenever P < 0.05.
FIGURE 1. A) Maternal serum levels of inhibin A in healthy pregnant women (○), partial hydatidiform mole (●) before and after evacuation, and in nonpregnant controls (▲) during the follicular phase of the menstrual cycle. Medians are indicated by horizontal bars. $P < 0.001$ vs. molar pregnancy before evacuation; $P < 0.001$ vs. values before evacuation. B) Maternal serum levels of activin A in healthy pregnant women (○), partial hydatidiform mole (●) before and after evacuation, and in nonpregnant controls (▲) during the follicular phase of the menstrual cycle. Medians are indicated by horizontal bars. $P < 0.001$ vs. molar pregnancy before evacuation; $P < 0.05$ vs. before evacuation. C) Maternal serum human chorionic gonadotropin levels in healthy pregnant women (○) and partial hydatidiform mole (●) before and after evacuation. Medians are indicated by horizontal bars. $P < 0.001$ vs. molar pregnancy before evacuation; $P < 0.05$ vs. before evacuation.
RESULTS
Molar pregnancies had inhibin A (P < 0.001), activin A (P < 0.001), and hCG (P < 0.001) levels significantly higher than normal healthy pregnant controls at the same stage of pregnancy (Table 1, Fig. 1). Hormone levels were significantly higher than pregnant controls at each evaluation before molar evacuation (Fig. 1).

DISCUSSION
Previous studies showed that serum immunoreactive inhibin levels in hydatidiform mole are significantly higher than in controls, but the assay did not distinguish between the various forms of inhibin-related proteins. Inhibin/activin subunits are localized in trophoblastic tumors, and the detection by immunohistochemistry of the inhibin subunits has been recently proposed as a useful immunohistochemical marker of trophoblastic neoplasia to be included in the diagnostic antibody panel in association with hCG. To our knowledge, the current study is the first conducted by using assays (ELISA) able to distinguish among inhibin A, inhibin B, and activin A, and showed that levels of serum inhibin A and activin A, but not inhibin B, are greatly increased in women with partial hydatidiform mole. This finding suggests roles for inhibin A and activin A measurements in diagnosing molar pregnancies, since those measurements resulted in 7-10 fold higher values than the 95% confidence interval of values in normal pregnant women without any considerable overlap with values found in normal pregnancies, unlike hCG measurements.

Also of clinical interest were the changes of inhibin A and activin A levels after molar evacuation. In fact, 10-15 days from the curettage, serum inhibin A (P < 0.001) and activin A (P < 0.05) concentrations in all six patients declined significantly to values similar to those in nonpregnant women (Table 1, Figs. 1A, 1B). Conversely, after molar evacuation, inhibin B levels did not change (data not shown), while hCG levels, despite significantly decreased (P < 0.05), remained far higher than in nonpregnant women (Table 1, Fig. 1C). The relevant fall of inhibin A and activin A levels after evacuation, other than supporting the mole as the source of these dimeric proteins, also suggests their clinical usefulness in identifying patients with spontaneous remissions after molar evacuation. Indeed, both inhibin A and activin A have a shorter half-life than hCG, as recently shown. In the current study, serum inhibin A and activin A levels after evacuation decreased to nonpregnant concentrations more rapidly than hCG levels, showing levels typical of fertile women, while serum hCG reached nonpregnant levels only about 10 weeks after the evacuation of hydatidiform mole. Currently, the persistence of

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STD: standard deviation; SEM: standard error of the mean; CI: confidence interval.
Before and after: levels in women with molar pregnancy before (performed with one day intervals) and after molar evacuation.
Pregnant: healthy pregnant controls.
Nonpregnant: healthy controls in the follicular phase of the menstrual cycle.
raised human hCG after evacuation of hydatidiform mole is used to identify patients with persistent trophoblastic disease before chemotherapy versus those patients with complete remission.\textsuperscript{1,2}

In conclusion, the current data strongly suggest that measurements of serum inhibin A and activin A may be of better value in diagnosing and following up molar pregnancies than hCG.

REFERENCES